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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Sarah Ferber
SERIAL NUMBER: 09/584,216 EXAMINER: Joseph T. Woitach
FILING DATE: May 31, 2000 ART UNIT: 1632
FOR: METHODS OF INDUCING REGULATED PANCREATIC HORMONE PRODUCTION IN NON-PANCREATIC ISLET TISSUES

MAIL STOP AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

not considered
JW 8/25/03

DECLARATION UNDER 37 C.F.R. § 1.132

I, Sarah Ferber hereby declare and state as follows:

1. I received my B.S., and Ph.D. degrees from the Technion-Biology Dept &Technion-School of Medicine in 1979 and 1988, respectively. I have been working in the field of Diabetes since 1988.

2. I am currently employed as a Scientist at Endocrine Institute, Sheba Medical Ctr., Tel-Hashomer, Israel, in the field of Diabetes Research. I am the owner and named inventor on the above-identified patent application and am intimately familiar with the methods of inducing pancreatic hormone production described by this application.

3. I am aware that, in a previous office (Office Action, Paper No. 13, mailed July 18, 2002), the Examiner has rejected claims directed to inducing pancreatic hormone expression in any cell type under 35 U.S.C. §112 contending that "the invention is based on the unexpected result that PDX expression in the liver is capable of inducing pancreatic hormone expression ... it is unclear that this unexpected result can be extended to any cell…[T]he specification fails to provide the necessary guidance to extend this unexpected result to other systems and other tissues for the expression of pancreatic genes." (Office Action, pp. 8-11).

4. I make this declaration to rebut the Examiner's assertion, with which I do not agree. It is my opinion that the specification as filed provides the necessary guidance to allow one of ordinary skill to use the methods recited by the newly added claims, which are directed to methods of inducing pancreatic hormone production in a skin cell. In view of the express statements in the specification regarding the induction of pancreatic hormone production in skin cells by using PDX-1, and in view of the experimental evidence that has been accumulated, it is my opinion that, at the filing date of this application, the ordinarily skilled artisan would have been able to routinely use the described methods to transdifferentiated skin cells to pancreatic cells without undue experimentation.

5. I have performed, or have had performed under my supervision, studies evaluating the transdifferentiation of skin cells (*i.e.*, keratinocytes) using AD-CMV-PDX. The methods used to perform these studies are described in the Appendix attached hereto.

6. The results of a first study are provided in Figure 1 in the Appendix. This study used four different treatment groups (K1-K4) to show insulin gene expression in keratinocytes treated with AD-CMV-PDX, EGF, KGF and NIC (K1) when compared to insulin gene expression in keratinocytes in the absence of AD-CMV-PDX. The results of this first study indicate that ectopic expression of PDX-1 induces skin cell transdifferentiation into insulin-, glucagon-, and somatostatin-producing cells. Specifically, the insulin gene in is translated into protein, which is then processed and secreted. The treatment used in this first study increases C-Peptide secretion (a product of pro-insulin processing) which suggests the induction of the relevant endopeptidases that are not otherwise active in normal, PDX-1 untreated Keratinocytes.

7. The results of a second study are provided in Figure 2 in the Appendix. This study used four different treatment groups (K1-K4) to show glucagon gene expression in keratinocytes treated with low levels of AD-CMV-PDX, EGF, and KGF (K4) when compared to glucagon gene expression in keratinocytes in the absence of AD-CMV-PDX. The results of this second study indicate that ectopic expression of PDX-1 induces skin to pancreas transdifferentiation. Furthermore, and importantly, EGF and KGF treatment alone was sufficient to induce skin cell transdifferentiation.

8. The results of a third study are provided in Figure 3 in the Appendix. This study used four different treatment groups (K1-K4) to show somatostatin gene expression in keratinocytes treated with l AD-CMV-PDX, EGF, KGF and NIC (K1) when compared to somatostatin gene expression in keratinocytes in the absence of AD-CMV-PDX. The results of this third study indicate that ectopic expression of PDX-1 induces skin cell transdifferentiation.

9. I understand that the specification contains explicit guidance about how to transdifferentiate liver cells (*see,* Examples 1-13 at pages 26-35 of the specification; and Example 16 at page 37 of the specification). Additionally, the specification teaches and discloses that various cell types capable of expressing PDX-1, including skin cells are useful in the methods of the invention. (*See* specification, page 6, lines 4-5; page 11, lines 9-10; and page 21, lines 12-28). In addition, I believe that the ordinarily skilled artisan would know how to actually carry out the steps of this invention according to the claimed methods. For this reason, in my view, it cannot be disputed that the ordinarily skilled artisan, with the specification in hand, would know how to use the invention as claimed. I do not believe that the Examiner disputes this; rather I believe that the Examiner is asking for additional proof that the ordinarily skilled artisan would be able to make and use the invention. I have provided that proof herein.

10. In addition, I further make this declaration to in support of new claims 48-50, which are directed to methods of inducing endogenous PDX-1 expression in cell. It is my opinion that the specification as filed provides the necessary guidance to allow one of ordinary skill to use the methods recited by these newly added claims. In view of the express statements in the specification regarding the induction of endogenous gene expression by using PDX-1, and in view of the experimental evidence that has been accumulated, it is my opinion that, at the filing date of this application, the ordinarily skilled artisan would have been able to routinely use the described methods to induce endogenous PDX-1 expression without undue experimentation.

11. I have performed, or have had performed under my supervision, studies evaluating the sustained developmental shift in the liver triggered by transient ectopic PDX expression.

12. The results of a first study are provided in Figure 4 in the Appendix. In this study, mice were treated by systemic delivery of recombinant adenovirus that directs expression of the rat

PDX-1 homologue. Specific oligonucleotide primers were used to distinguish between the ectopic PDX-1 transgene (rat) mRNA (cDNA) and the endogenous mouse mRNA. PCR analysis of DNA samples isolated from liver of *Ad-CMV-PDX-1* treated mice demonstrated that the virally encoded transgene disappeared between 30 and 56 days after adenovirus injection. (Figure 4A). The ectopic rat PDX-1 expression parallels the observed presence of delivered viral DNA in liver. (Figure 4B). Moreover, the only homologue of PDX-1 that was expressed in treated livers for the whole duration of the experiment was the endogenous and otherwise silent mouse homologue. (Figure 4B). Endogenous PDX-1 expression is exclusive to mice that received the rat PDX-1 transgene, and was evident in 75% of ectopic PDX-1 treated mice (21 out of 28 mice), and in none of the 25 control-treated livers that were analyzed in this first study.

13. In a second study, the identity and quantities the relative levels of mouse versus rat PDX-1 gene expression in liver, as a function of time after the initial treatment, was analyzed using real time PCR. PCR was performed using identical conditions (but different primers) and was normalized to β-actin within the same samples. The mRNA encoding the ectopic rat PDX-1 is maximal at 5 days, drops by 85% at day 30 and disappears thereafter. By contrast, the endogenous mouse PDX-1 is expressed at substantial levels for the whole duration of the experiment. (Figure 4C)

14. The results of these studies taken together indicate an auto-induction of the endogenous and otherwise silent PDX-1 in liver, which suggest a mechanistic explanation for the long-lasting mode of liver to pancreas transconversion process.

15. I understand that the specification contains explicit guidance about how to transdifferentiate liver cells (*see,* Examples 1-13 at pages 26-35 of the specification; and Example 16 at page 37 of the specification). Additionally, the specification teaches and discloses that ectopic expression of PDX-1 induces endogenous and otherwise silent genes. (*See* specification, page 36, lines 16-24; and page 20, lines 5-6). In addition, I believe that the ordinarily skilled artisan would know how to actually carry out the steps of this invention according to the claimed methods. For this reason, in my view, it cannot be disputed that the

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ordinarily skilled artisan, with the specification in hand, would know how to use the invention as claimed

16. For all the foregoing reasons, I believe that the Examiner should allow the pending claims.

17. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Sarah ferber
Sarah Ferber

Signed at Tel-Hashomer, Israel
this 11 day of June 2003

Appendix

PDX-1 and Growth factors induced transdifferentiation in primary culture of human keratinocytes.

Cell Culture

Keratinocyte cultures were initiated from small biopsy specimens (2-4cm^2) of split-thickness skin. After overnight (ON) incubation in trypsin-EDTA the epidermis was separated and epithelium disaggregated in trypsin-EDTA to form single cells suspension. The cell suspension was cultured in Keratinocyte Medium (Nature 265: 421-4, 1977), and the cell suspension was attached to falcon culture plates and used at passages 2-5. When cells reached 70% confluency, they were treated by the indicated treatments described below for 48-96 hours.

Gene Expression

Gene expression analyses were performed using Taqman real time PCR (ABI).

Cell Treatment

K1: EGF+KGF+NIC+PDX-1(100 moi) K2: EGF+KGF+NIC+PDX-1(10 moi)

K3: EGF+KGF K4: EGF+KGF+RGCI

At all treatments: EGF, KGF are 20ng/ml; NIC: 10ng/ml

Controls

The control cells were treated with a non-relevant, Ad-CMV-Hinsulin, recombinant adenovirus that carried the expression of the human insulin gene under the control of the CMV promoter. RGCI is a bifunctional recombinant adenovirus construct--Ad-CMV-PDX-1-RIP-GFP that identified cells that had undergone PDX-1 mediated transdifferentiation toward insulin gene expression. PDX-1 expression in this virus was driven by CMV promoter, whereas GFP expression was driven by the tissue specific promoter for insulin (RIP).

Results

In the treatments K1-K4, the endogenous otherwise silent pancreatic genes were expressed in keratinocytes. Interestingly, glucagon gene expression was induced by low levels of PDX-1 (K4) and importantly, by EGF+KGF treatment alone, with no need for ectopic PDX-1. (Fig. 2)